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Lecithin based nanoemulsions: A comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behaviour and skin permeation

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ABSTRACT

Charged drug delivery systems are interesting candidates for the delivery of drugs through skin. In the present study, it was possible to create negatively and positively charged oil/water nanoemulsions by using sucrose laureate and polysorbate 80 as non-ionic surfactants. The positively charged nanoemulsions were generated by adding cationic phytosphingosine (PS). The relationship between the physicochemical properties of the nanoemulsions was shown by particle size and zeta potential measurements. These properties were dependent on the type of non-ionic surfactant and the concentration of PS. Furthermore the cationic PS had a positive impact on the skin permeation rates (flux) of the incorporated model drugs fludrocortisone acetate and flumethasone pivalate. An enhancement factor between 1.1 and 1.5 was obtained in relation to the control. The interaction of pre-impregnated porcine skin with positively and negatively charged nanoemulsions was confirmed by DSC analysis. The generated DSC-curves showed a slight difference in the phase transition temperature assigned to the characteristic lipid transition. However, it was not possible to assign the effect to one of the ingredients in the multicomponent system.

1. Introduction

Nanoemulsions possess numerous advantages including the possibilities of controlled drug release and drug targeting, and the incorporation of a great variety of therapeutic actives (Calderilla-Fajardo et al., 2006). Usually the droplets achieve a size range between 50 and 200 nm. Unlike microemulsions they are stabilised by non-ionic surfactants and/or polymers exhibiting a steric stabilisation effect. Due to their small particle sizes, nanoemulsions are able to penetrate easily through the skin layers and enhance skin penetration of incorporated drugs (Tadros et al., 2004). Another important advantage is the low surfactant concentration compared to microemulsions (Tadros et al., 2004). Several preparation techniques such as spontaneous emulsification, phase inversion temperature (PIT) emulsification, phase inversion composition and high-pressure homogenisation are known. The colloid-chemical structure depends thereby on these processes (Sonneville-Aubrun et al., 2004).

Due to their effectiveness for drug solubilization nanoemulsions offer an alternative for the administration of poorly water soluble drugs. This leads to improved efficacy and compliance because of reduced side effects (Kelmann et al., 2007). Nevertheless the use of safe and skin friendly ingredients is desirable for such colloidal formulations. As already reported, Lecithin, a natural mixture can fulfil these requirements, generally it is non-irritating and non-sensitizing for animal and human skin (Fiume, 2001; Paolino et al., 2002). Moreover lecithin compounds present an affinity to cellular membranes thus leading to an increased absorption of several drugs (Paolino et al., 2002). Sucrose esters are also non-irritant and biodegradable surfactants and therefore suitable for pharmaceutics and cosmetics. As previously published sucrose esters are promising candidates for enhanced drug permeability through skin showing lipid extraction and fluidization of the stratum corneum. Among the group of sucrose esters sucrose laureate has already been used to enhance skin permeability (Cazares-Delgadillo et al., 2005; Calderilla-Fajardo et al., 2006).

Cationic compounds can also have a positive effect on skin permeation, since the skin carries a negative surface charge due to the negatively charged residues of proteins (Yilmaz and Borchert, 2005). One candidate for this purpose could be the physiological phytosphingosine (PS), a natural compound in the human body as well as in the stratum corneum. PS exhibited anti-inflammatory and antimicrobial effects both being interesting for topical use (Yilmaz and Borchert, 2005; Farwick et al., 2007).

The aim of the present study was to design physically stable nanoemulsions consisting of lecithin, vitamin E, PS, sucrose lau-

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reate and polysorbate 80, respectively. After the physicochemical characterisation by particle size and zeta potential measurements the influence of surfactant type and PS on skin permeation rates of the model drug fludrocortisone acetate was investigated. In order to confirm the influence of the cationic PS on skin diffusion additional permeation studies were performed using flumethasone pivalate in the polysorbate 80 nanoemulsion. Furthermore information about the possible interactions with skin lipids should be provided by DSC measurements of porcine skin samples after exposure to the various nanoemulsion formulations.

2. Materials and methods

2.1. Materials

Fludrocortisone acetate (CAS: 514-36-3) was purchased from Sigma–Aldrich (St. Louis, USA). Flumethasone pivalate (CAS: 2002-29-1) was supplied from Kemprotec (Middlesbrough, UK). Phytosphingosine (PS) was kindly provided by Degussa (Cosmoferm BV, NL). Lipoid S-75 was obtained from Lipoid GmbH (Ludwigshafen, Germany), containing 69.9% phosphatidylcholine, 8.4% phosphatidylethanolamine, 2.2% lysophosphatidylcholine according to manufacturers specifications. Sucrose laureate (SL, Ryoto Sugar Ester® L-1695) was gently donated by Mitsubishi-Kasei Food Corporation (Tokyo, Japan). Polysorbate 80 and the antioxidant α-tocopherol were supplied by Pauli GmbH & Co. KG (Vienna, Austria). PCL-liquid (Etylhexanoate) was purchased from Symrise GmbH & Co. KG (Holzminden, Germany). All other chemicals used were of analytical reagent grade and used as received without any further purification.

2.2. Formulations

The nanoemulsions were prepared by mixing the separately prepared aqueous and oily phases. The aqueous phase containing sucrose laureate or polysorbate 80 and distilled water was heated up to 50 °C under slight mixing. When PS was incorporated it was dissolved in the oil phase, containing PCL-liquid, Lipoid S75, α-tocopherol and model drug (1%). The two phases were merged and pre-homogenised for 3 min with an ultra-Turrax (Omni 500) at 2500 rpm and continuously stirred for 24 h at room temperature. Afterwards the raw formulation was pre-homogenised once more for 3 min at 50 °C and further homogenised with a high-pressure homogeniser (EmulsiFlex-C3, Avestin) for 12 homogenisation cycles at 600 bars. In Table 1 all formulations performed with code names and their percentage compositions are presented. The meaning of the code names is detailed below Table 1.

2.3. Nanoemulsion characterisation

2.3.1. Particle size

The mean particle size and size distribution were determined by photon correlation spectroscopy with a Zetasizer Nano ZS (Malvern, UK) at 25 °C. Each nanoemulsion was diluted to the appropriate concentration with distilled water to weak opalescence. The size distribution was represented by the polydispersity index (PDI) values. Thereby PDI values lower than 0.25 indicate a close size distribution providing good stability of nanoemulsions due to the reduced Ostwald ripening (Yilmaz and Borchert, 2005). The measurements were performed using a He–Ne laser at 633 nm. The diameters were determined immediately after nanoemulsion preparation and checked over 10 weeks during storage at room temperature.

2.3.2. Zeta potential

The surface charge was analysed using a Zetasizer Nano ZS (Malvern, UK) at 25 °C by measuring the zeta potential (ZP) of the preparations. For this purpose the samples were diluted with distilled water. The ZP characterises the surface charge of particles and thus gives information about repulsive forces between particles and droplets. Absolute higher values than 30 mV usually indicate good stability of the system (Müller, 1996). Also ZP values were measured after nanoemulsion preparation and checked over 10 weeks during storage at room temperature.

2.3.3. Skin permeation experiments

In vitro permeation studies with porcine abdominal skin were performed using Franz-type diffusion cells. Porcine abdominal skin was chosen as a model based on the similarity in permeability and morphology to human skin (Cazares-Delgadillo et al., 2005). Abdominal porcine skin was shaved and then prepared with a dermatome (GB 228R, Aesculap) set at 1 mm and afterwards stored in a freezer at −20 °C until use. Two hours prior to the experiment the samples were thawed.

The skin was clamped between the donor and the receptor chamber of Franz-type diffusion cells having a permeation area of 1.13 cm^2. The receptor compartment was filled with 2 ml of 0.012 M phosphate buffer (pH, 7.4). The recently investigated solubility of the model drugs in 0.012 phosphate buffer (Hoeller and Valenta, 2007) guaranteed sink conditions. The diffusion cells were thermostated at skin surface temperature of 32 °C and stirred by magnetic bars. The formulation (0.6 g) was gently placed in the donor chamber. Samples of 200 μl were removed at defined time intervals for analysis and replaced immediately by an equal volume of fresh buffer. At least three parallel experiments were performed for each formulation. Each 20 μl of the samples were analysed for their drug content by HPLC (PerkinElmer, US) consisting of an

Table 1
Composition of the nanoemulsion formulations and abbreviations.

Excipients	Nanoemulsion composition (% w/w)					
	NL	NL-0.4PS	NL-0.6PS	NT	NT-0.4PS	NT-0.6PS
Lipid phase						
PCl-liquid	20	20	20	20	20	20
Lipoid S75	4	4	4	4	4	4
Alpha-tocopherol	1	1	1	1	1	1
Phytosphingosine (PS)	–	0.4	0.6	–	0.4	0.6
Fludrocortisone acetate	1	1	1	1	1	1
Aqueos phase						
Sucrose laureate L-1695	1	1	1	–	–	–
Polysorbate 80	–	–	–	1	1	1
Distilled water to	100	100	100	100	100	100

Abbreviations: NL, sucrose laureate nanoemulsion without PS; NL-0.4PS, sucrose laureate nanoemulsion with 0.4% PS; NL-0.6PS, sucrose laureate nanoemulsion with 0.6% PS; NT, polysorbate 80 nanoemulsion without PS; NT-0.4PS, polysorbate 80 nanoemulsion with 0.4% PS; NT-0.6PS, polysorbate 80 nanoemulsion with 0.6% PS.

Table 2
Physicochemical properties of the nanoemulsions after 12 homogenisation cycles with a high pressure homogeniser.

Property	Sucrose laureate nanoemulsion			Polysorbate 80 nanoemulsion		
	NL	NL-0.4PS	NL-0.6PS	NT	NT-0.4PS	NT-0.6PS
Particle size (nm)	161 ± 0.7	215 ± 2.8	254 ± 2.2	170 ± 3.8	216 ± 26.6	176 ± 2.1
Zeta potential (mV)	−62 ± 0.4	+46 ± 0.4	+48 ± 0.7	−55 ± 0.7	+45 ± 0.7	+48 ± 1.1
PDI	0.12–0.22	0.22–0.25	0.06–0.10	0.15–0.18	0.13–0.18	0.10–0.14
pH	7.21 ± 0.05	8.96 ± 0.19	6.69 ± 0.12	5.3 ± 0.08	6.7 ± 0.06	7.1 ± 0.12

PDI, polydispersity index; PS, phytosphingosine.
Indicated values are the average (±S.D.) of three experiments.

automatic auto sampler ISS-200, a pump and an UV-diode array detector. A previously reported method was used (Cisternino et al., 2003). Briefly, the used column was a Nucelosil 100–5 C18 column (240 mm × 4 mm; ARC-Seibersdorf, Austria) and the mobile phase consisted of acetonitrile and water (40:60, v/v), respectively. The detection wavelength was set at 240 nm and the flow rate was 0.8 ml/min for fludrocortisone acetate and 1.0 ml/min for flumethasone pivalate. Calibration curves were calculated on the basis of peak area measurements of the used standard solutions. The calibration curves had a correlation coefficient of 0.9998 and 0.9991, respectively. The concentration range of the standard solutions was between 3.87 and 124 μg/ml for fludrocortisone–acetate and between 3.48 and 110.9 μg/ml for flumethasone pivalate.

Additionally, the steady state flux J [$\mu\,cm^{-2}\,h^{-1}$] across the skin was calculated from the slope of linear portion of the cumulative amount permeated through the porcine skin per unit area versus time plot.

2.3.4. Differential scanning calorimetry (DSC)

DSC (PerkinElmer DSC-7) was used to characterise the thermal transition of porcine skin samples of one individual that were either pre-impregnated with water or positively and negatively charged nanoemulsion formulations. The method used is already described (Valenta et al., 2001). Briefly, prior to DSC measurements about 25 mg of porcine skin were impregnated with 2 ml of water or differently charged nanoemulsions in a petri dish for 12 h. Porcine skin impregnated with water served as control. Afterwards the skin samples were blotted dry and then hermetically sealed within an aluminium holder and heated from 30 to 120 °C at a heating rate of 5 °C/min. The generated DSC-curves were compared to the control in terms of transition temperature and linear onset.

Gravimetric analysis of all samples prior to DSC experiments showed average water content of about 72.91% (w/w) ±5.83.

2.3.5. Statistical data analysis

Results are expressed as the means of minimum three experiments ± S.D. Statistical data analysis was performed using the t-test with $P < 0.05$ as a minimal level as significance.

3. Results

3.1. Formulations

A visual inspection of the different formulations immediately after production showed liquid, whitish and well distributed nanoemulsions in all cases.

3.2. Nanoemulsion characterisation

The physicochemical properties of the formulations after 12 homogenisation cycles are shown in Table 2. As seen, the mean particle size depended on the type of surfactant and was strongly influenced by the presence of PS. In the sucrose laureate nanoemulsion the addition of PS increased the particle sizes in following manner: the higher the PS content, the higher the particle sizes of the nanoemulsions. In contrast to this in the polysorbate 80 nanoemulsion an increase from 0.4% to 0.6% PS decreased the particle size from 216 to 176 nm, which is nearly the size of the PS free polysorbate 80 nanoemulsion. As expected the surface charge of the nanoemulsions shifted from negative to positive by adding PS. The absolute values being above 45 mV indicate a good electrochemical stability. The PDI values lower than 0.25 pointed to close size distributions.

A set of experiments was performed over a pH-range from 5 to 8. Whereas the PS free nanoemulsions with sucrose laureate and polysorbate 80 showed a constant high ZP and a uniform particle size over the whole pH-range, the PS containing nanoemulsions exhibited a completely different behaviour (Fig. 1a and b). When

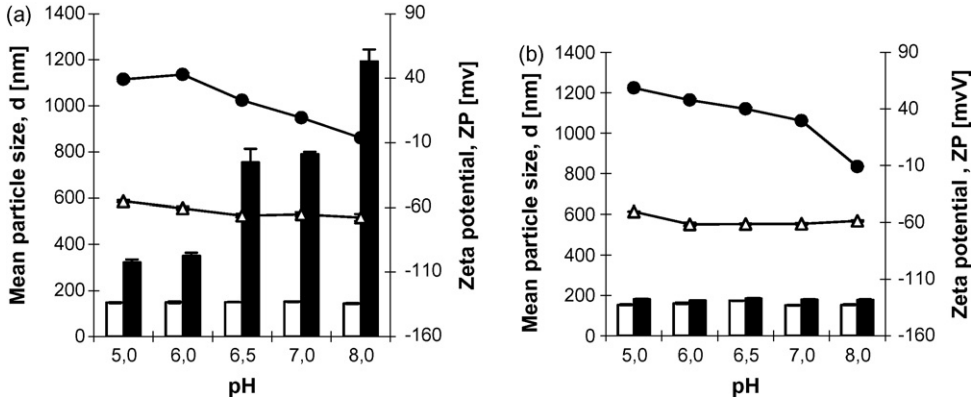

Fig. 1. Effect of pH on the mean particle size and zeta potential (ZP) from sucrose laureate and polysorbate 80 nanoemulsions. Indicated values are means (±S.D.) of three experiments. (a) Sucrose laureate nanoemulsions—white bars: mean particle size of NL; black bars: mean particle size of NL-0.6PS; (▲) zeta potential of NL; (●) zeta potential of NL-0.6PS. (b) Polysorbate 80 nanoemulsions—white bars: mean particle size of NT; black bars: mean particle size of NT-0.6PS; (▲) zeta potential of NT; (●) zeta potential of NT-0.6PS.

0.6% PS was incorporated in sucrose laureate nanoemulsion (Fig. 1a, black bars) the particle size increased dramatically up to pH 6.5. The biggest particle sizes of about 1200 nm were seen at pH 8.0. When the pH value was decreased from 8 to 5, the ZP values increased from about −10 mV to above +40 mV in the nanoemulsions with PS. In case of the polysorbate 80 nanoemulsion (Fig. 1b, black bars) the particle size was slightly increased but uniformly also by addition of 0.6% PS over the whole observed pH-range, which is an indication for a higher stability. The ZP changes followed the same pattern as in the sucrose laureate nanoemulsion at higher pH to negative.

The stability assessments of the tested nanoemulsions are shown in Fig. 2 a and b. As seen, a constant ZP and uniform particle size over an observation period of 10 weeks are indicated in the PS free nanoemulsions (Fig. 2a and b, white bars) as well as in the PS containing nanoemulsion with polysorbate 80 (Fig. 2a, grey and black bars). Although, the particle size is slightly increased in the PS containing preparation the nanoemulsion can be quoted as physically stable. In general, the polysorbate 80 nanoemulsion show particle sizes in the range from 165 to 250 nm independent of the surface charge.

In contrast phase separation was observed in the sucrose laureate nanoemulsion containing 0.4% PS after 10 days. On the contrary the formulation with 0.6% PS exhibited improved physical stability. However, after 7 weeks of storage a pronounced particle agglomeration occurred simultaneously with a dramatic decrease of the ZP (Fig. 2b, black bars).

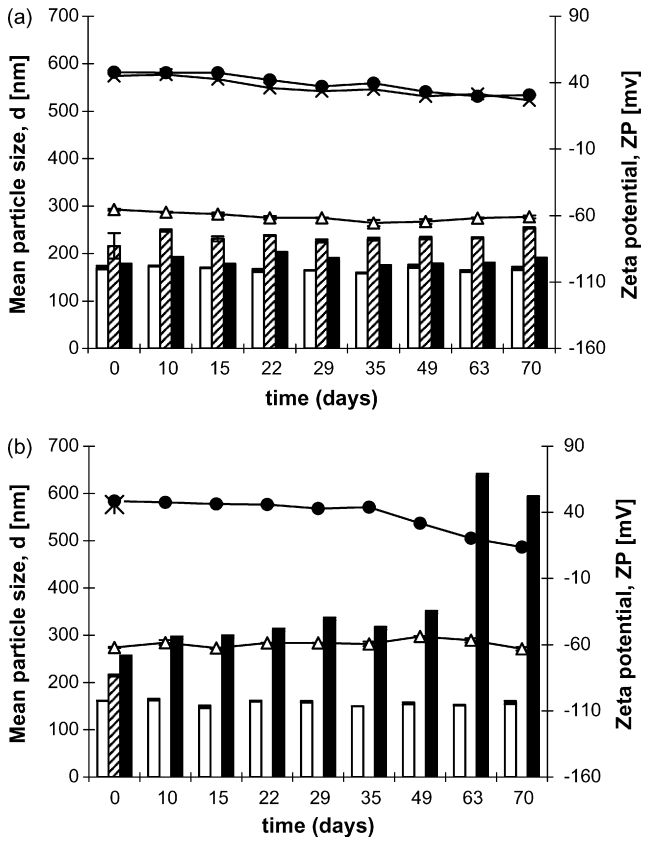

Fig. 2. Mean particle size and zeta potential (ZP) after storage of sucrose laureate and polysorbate 80 nanoemulsions over an observation period of 10 weeks. Indicated values are means (±S.D.) of three experiments. If no bars are shown, phase separation occurred. (a) Polysorbate 80 nanoemulsions—white bars: mean particle size of NT grey lined bars: mean particle size of NT-0.4PS black bars: mean particle size of NT-0.6PS; (▲) zeta potential of NT; (X) zeta potential of NT-0.4PS; (●) zeta potential of NT-0.6PS. (b) Sucrose laureate nanoemulsions—white bars: mean particle size of NL grey lined bars: mean particle size of NL-0.4PS black bars: mean particle size of NL-0.6PS; (▲) zeta potential of NL; (X) zeta potential of NL-0.4PS; (●) zeta potential of NL-0.6PS.

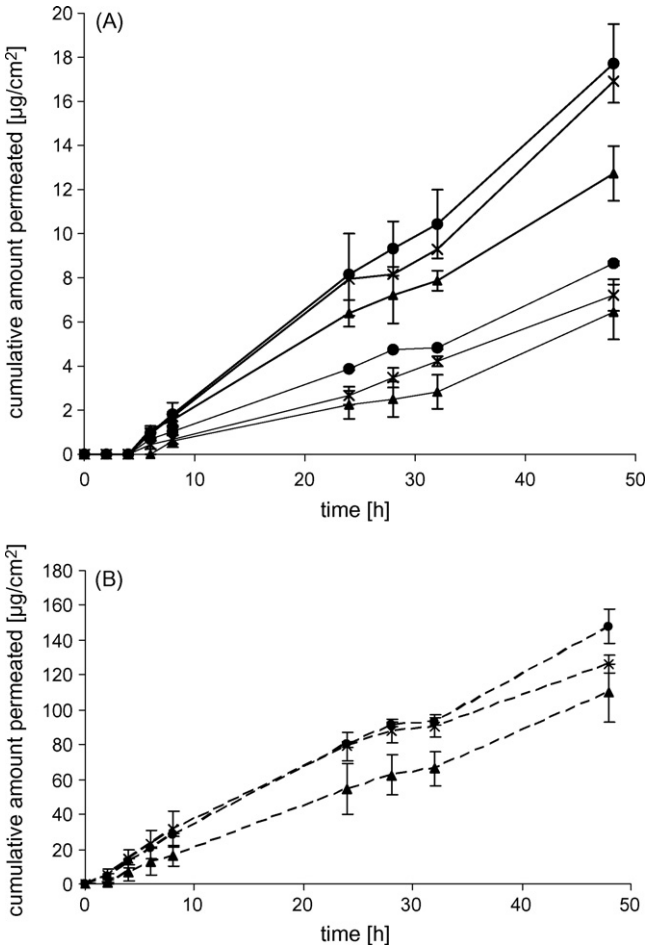

Fig. 3. Permeation profiles of fludrocortisone acetate (A) and flumethasone pivalate (B) incorporated in positively and negatively charged nanoemulsions through porcine skin determined by HPLC. Indicated values are means (±S.D.) of three experiments. (A) Full line (●) NT-0.6PS; full line (X) NT-0.4PS; full line (▲) NT; dashed line (- - ● - -) NL-0.6PS; dashed line (- - x - -) NL-0.4PS; dashed line (- - ▲ - -) NL. (B) Dashed line (– – ● – –). NT-0.6PS; dashed line (– – x – –) NT-0.4PS; dashed line (– – ▲ – –) NT.

3.3. Skin permeation experiments

Firstly, in standard diffusion experiments the skin permeation rates (flux) of the model drug fludrocortisone acetate were investigated. As seen in Fig. 3A two groups can be distinguished: one group having lower fluxes of fludrocortisone acetate is based on the nanoemulsion with sucrose laureate and a second group exhibiting higher diffusion rates is based on polysorbate 80 nanoemulsion. Moreover, PS was able to increase the skin permeation rates of fludrocortisone acetate compared to the control independent of the formulation. The addition of 0.6% PS led to an increase in the permeation rates of 1.5 times from sucrose laureate nanoemulsion and 1.4 times of the polysorbate 80 nanoemulsion compared to the control (Table 3).

To confirm the influence of PS on the skin rates a set of formulation with the polysorbate 80 nanoemulsion containing flumethasone pivalate as other model drug was investigated. Again the positive impact of the cationic PS on the flux was demonstrated, although to a lesser extent than in the experiments using fludrocortisone acetate (Table 3 and Fig. 3B). The enhancement factor for flumethasone pivalate by the addition of 0.6% PS was 1.34.

Table 3
Skin permeation rates (flux) of fludrocortisone acetate and flumethasone pivalate in different nanoemulsions and influence of phytosphingosine (PS) through porcine skin, $n = 3$.

Model drug	Nanoemulsion	Flux (J, $\mu g\,cm^{-2}\,h^{-1}$)	Enhancement factor of PS compared to control
Fludrocortisone acetate	NL	0.126 ± 0.027	Control
	NL-0.4PS	0.150 ± 0.010	1.1
	NL-0.6PS	0.189 ± 0.012*	1.5*
	NT	0.263 ± 0.043	Control
	NT-0.4PS	0.353 ± 0.018*	1.3*
	NT-0.6PS	0.377 ± 0.038*	1.4*
Flumethasone pivalate	NT	2.290 ± 0.313	Control
	NT-0.4PS	2.698 ± 0.117	1.18
	NT-0.6 PS	3.073 ± 0.104*	1.34*

* Indicate a statistical significance, $p < 0.05$.

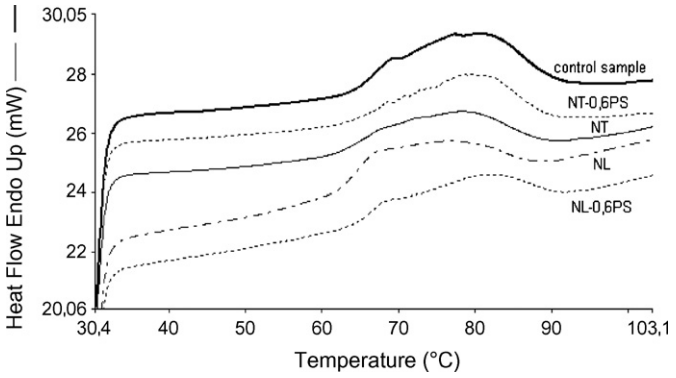

Fig. 4. Differential scanning calorimetry (DSC) analysis of porcine skin treated with negatively and positively charged nanoemulsions. Gravimetric analysis of the samples prior to DSC studies showed average water content of about 73% (w/w). Graphs are representative of $n \geq 4$ replicate samples.

3.4. Differential scanning calorimetry

To further explore the effect of different nanoemulsions on lipid components of porcine skin DSC studies were conducted. All samples were taken from the same piece of porcine skin. In literature data it was proposed that porcine transitions occurred near 60, 70 and 100 °C in hydrated samples, which were due to intercellular lipid, protein-intercellular lipid and keratin, respectively (Potts, 1989; Valenta et al., 2001; Kim et al., 2008). In our case porcine skin pre-impregnated with water for 12 h showed an endothermic transition peak T_m occurring near 80 °C. The pre-impregnation of porcine skin with differently charged nanoemulsions resulted in a slight difference of T_m (Fig. 4 and Table 4). The maximal thermal transition is shifted from about 80 to 74 °C after treatment with negatively charged nanoemulsions independent of the non-ionic surfactant. Impregnation of porcine skin with positively charged nanoemulsions resulted in a T_m shift from 80 to 76 °C with the polysorbate 80 containing nanoemulsion, whereas no influence of the positively charged sucrose laureate nanoemulsion was observed.

Table 4
Thermal changes following pre-impregnation of porcine skin with positively and negatively charged nanoemulsions. Control: porcine skin pre-impregnated with water for 12 h, $n \geq 4$.

Treatment	DSC T_m (°C)	Linear onset (°C)
Control	80.01 ± 0.45	65.29 ± 0.20
NL	74.38 ± 4.53	65.54 ± 5.17
NL-0.6PS	80.66 ± 0.31	64.14 ± 0.19
NT	74.90 ± 2.95	64.47 ± 2.72
NT-0.6PS	76.85 ± 3.81	66.52 ± 2.35

T_m: Temperature of the transitions maximum.

4. Discussion

This study investigated differently charged nanoemulsions containing either sucrose laureate or polysorbate 80 in terms of particle size and zeta potential. It is known that non-ionic surfactants stabilise emulsion due to a strong steric repulsion mechanism (Piemi et al., 1999). As the results indicate polysorbate 80 improved the physicochemical stability of the formulation in comparison to the sucrose laureate nanoemulsions. Due to ethylene oxide groups and the long hydrocarbon chain a stronger steric stabilisation effect can be achieved. Therefore mean particle size and zeta potential values of the polysorbate 80 containing nanoemulsions were physically stable over 10 weeks showing no flocculation, creaming, coalescence and Ostwald ripening.

Additionally we tested the hypothesis that PS, a known sphingoid base, can increase skin permeability by its cationic charge on the negatively charged skin (Yilmaz and Borchert, 2005). The obtained data confirmed the pH dependent physicochemical properties of the tested PS nanoemulsion. The electrical surface charge of the particles is produced by ionisation of PS forming an interfacial film. It depends mainly on the extent of the ionisation of PS (Fig. 1a and b).

Percutaneous penetration implies several steps. The release of a drug through skin will depend on the physicochemical properties of the drug itself combined with the influence of the vehicle to alter the drug penetration profile (Piemi et al., 1999; Cazares-Delgadillo et al., 2005). The positively charged nanoemulsions containing PS were found to be more effective in terms of skin diffusion of fludrocortisone acetate and flumethasone pivalate through porcine skin than the negatively charged ones (Table 3 and Fig. 3A and B).

As mentioned the interaction of nanoemulsions with skin depends upon a number of factors including also the electrical charge of the droplets. The results obtained suggest that positively charged particles of the nanoemulsion systems are able to carry efficiently fludrocortisone acetate and flumethasone pivalate into the skin and subsequently promote the penetration of the drugs through skin. The degree of skin binding is probably more important with the positively charged particles than with the negatively one as it is known that the skin is negatively charged at neutral pH (Conrads and Zahn, 1987). This hypothesis is supported additionally by the results of Piemi et al. (1999). It can therefore be deduced that the binding of the charged particles to the skin can be attributed to a specific electrostatic interaction due to the positive charge of the particles in case of the phytosphingosine containing nanoemulsions (Piemi et al., 1999). Nevertheless, more penetration studies need to be performed to confirm the influence of the charge of the vehicle and the binding skin process, especially in the case of positively or

negatively charged nanoemulsions in order to establish a possible structure-binding relationship.

Moreover the penetration enhancement of drugs is closely related to the nature of the surfactants used in the formulation. Many penetration enhancers are capable of inserting themselves between the hydrophobic tails of the bilayer, thus disturbing their packing, increasing their fluidity, and subsequently, leading to an easier diffusion of lipid penetrants. Sucrose laureate interferes with their long hydrocarbon chain between the lipophilic tails allowing the sucrose ring to interact with the polar lipid head groups (Calderilla-Fajardo et al., 2006). There are two possible mechanisms of penetration enhancement suggested for polysorbate 80. On one hand it penetrates into the intercellular regions of stratum corneum, increases fluidity and eventually solubilises and extracts lipid components, and on the other hand an interaction and binding with keratin filaments may result in a disruption within the corneocytes. In this case keratin filaments and their associated water molecules could be disrupted, whereas a solubilising ability of the aqueous layer could result and an allowed drug transport through the corneocytes might be possible (Nokhodchi et al., 2003). Consequently one reason for the improved skin permeation of fludrocortisone acetate caused by using polysorbate 80 will be the protein domains involved.

In DSC-studies a slight difference was observed between the skin samples pre-impregnated with differently charged nanoemulsions and the water-impregnated control. The decreased transition temperatures showed an interaction with lamellar skin lipids structure at temperature near 60–80 °C indicating a higher fluidity of skin. Due to the higher skin permeation of PS containing nanoemulsions we expected a further shift of the transition temperature to lower values. However the opposite was observed (Table 4). Therefore, the higher skin permeation might be caused by additional other mechanisms than by skin lipid interactions. It is also known that the water content plays a major role in the transition temperature of lipids and proteins (Potts, 1989). For that reason it was seen to it that all samples had equal water content prior to DSC-measurements.

5. Conclusion

Overall, we succeeded in creating nanoemulsions with the skin friendly mild surfactant sucrose laureate and in enhancing the skin fluxes of the model drug fludrocortisone acetate and flumethasone pivalate with PS. In addition the nanoemulsion with conventional polyoxyethylene derivatives exhibited higher diffusion rates and a prolonged physicochemical stability. Furthermore PS is an interesting multifunctional additive for drug delivery systems due to its enhancement effect on skin permeation and above all its hydrating and anti-inflammatory power.

References

Calderilla-Fajardo, S.B., Cazares-Delgadillo, J., Villalobos-Garcia, R., Quintanar-Guerrero, D., Ganem-Quintanar, A., 2006. Influence of sucrose esters on the in vivo percutaneous penetration of octyl methoxycinnamate formulated in nanocapsules, nanoemulsion and emulsion. Drug Dev. Ind. Pharm. 32, 107–113.

Cazares-Delgadillo, J., Naik, A., Kalia, Y.N., Quintanar-Guerrero, D., Ganem-Quintanar, A., 2005. Skin permeation enhancement by sucrose esters: a pH-dependent phenomenon. Int. J. Pharm. 297, 204–212.

Cisternino, S., Schlatter, J., Saulnier, J.L., 2003. Stability of Fludrocortisone-acetate solutions prepared from tablets and powder. Eur. J. Pharm. Biopharm. 55, 209–213.

Conrads, A., Zahn, H., 1987. A study of the interaction of sodium dodecyl sulphate with the proteins of human heel stratum corneum. Int. J. Cosmet. Sci. 9, 29–46.

Farwick, M., Watson, R.E.B., Rawlings, A.V., Wollenweber, U., Lersch, P., Bowden, J.J., Bastrilles, J.Y., Griffiths, C.E.M., 2007. Salicyloly-phytosphingosine: a novel agent for the repair of photoaged skin. Int. J. Cosmet. Sci. 29, 319–329.

Fiume, Z., 2001. Final report on the safety assessment of lecithin and hydrogenated lecithin. Int. J. Toxicol. 20, 21–45.

Hoeller, S., Valenta, C., 2007. Effect of selected fluorinated drugs in a "ringing" gel on Rheological behaviour and skin permeation. Eur. J. Pharm. Biopharm. 66, 120–126.

Kelmann, R.G., Kuminek, G., Teixeira, H.F., Koester, L.S., 2007. Carbamazepine parenteral nanoemulsions prepared by spontaneous emulsification process. Int. J. Pharm. 342, 231–239.

Kim, Y.-C., Park, J.-H., Ludovice, P.J., Prausnitz, M.R., 2008. Synergistic enhancement of skin permeability by N-lauroylsarcosine and ethanol. Int. J. Pharm. 352, 129–138.

Müller, R.H., 1996. Zetapotential und Partikelladung in der Laborpraxis. Paperback APV, Band 37. Wissenschaftliche Verlagsgesellschaft mbH Stuttgart.

Nokhodchi, A., Shokri, J., Dashbolaghi, A., Hassan-Zadeh, D., Ghafourian, T., Barzegar-Jalali, M., 2003. The enhancement effect of surfactants on the penetration of lorazepam through rat skin. Int. J. Pharm. 250, 359–369.

Paolino, D., Ventura, C.A., Nistico, S., Puglisi, G., Fresta, M., 2002. Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo human skin tolerability. Int. J. Pharm. 244, 21–31.

Piemi, M.P.Y., Korner, D., Benita, S., Marty, J.P., 1999. Positively and negatively charged submicron emulsions for enhanced topical delivery of antifungal drugs. J. Control Rel. 58, 177–187.

Potts, R.O., 1989. In: Hadgraft, J., Guy, R.H. (Eds.), Transdermal Drug Delivery, vol. 35. Marcel Dekker, New York, pp. 23–57.

Sonneville-Aubrun, O., Simonnet, J.T., L' Alloret, F., 2004. Nanoemulsions: a new vehicle for skincare products. Adv. Colloid Interface Sci. 108–109, 145–149.

Tadros, T., Izquierdo, P., Esquena, J., Solans, C., 2004. Formation and stability of nanoemulsions. Adv. Colloid Interface Sci. 108–109, 303–318.

Valenta, C., Nowak, M., Hadgraft, J., 2001. Influence of phloretin and 6-ketocholestanol on the permeation of progesterone through porcine skin. Int. J. Pharm. 217, 79–86.

Yilmaz, E., Borchert, H.-H., 2005. Design of a phytosphingosine-containing, positively-charge nanoemulsion as a colloidal carrier system for dermal application of ceramides. Eur. J. Pharm. Biopharm. 60, 91–98.